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Effects of Continuous Zinc Exposure on Sockeye Salmon During Adult-to-Smolt Freshwater Residency

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Effects of Continuous Zinc Exposure on Sockeye Salmon During Adult-to-Smolt Freshwater Residency

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ABSTRACT

This chronic zinc toxicity test with sockeye salmon (*Oncorhynchus nerka*) consisted of a 3-mo adult exposure followed by an 18-mo exposure of embryonic through smolt stages. The zinc concentrations utilized were 30 to 112 μg/liter during the adult-to-smolt exposure period, and an additional 242 μg/liter concentration was used for the embryo-to-smolt exposure period. These zinc concentrations produced no adverse effects on survival, fertility, fecundity, growth, or on the subsequent survival of smolts transferred to seawater. Exposure to 242 μg/liter produced sufficient acclimation to markedly decrease acute mortality at zinc levels lethal to unacclimated sockeye salmon juveniles. The 112- and 242-μg/liter concentrations were 0.15 and 0.32, respectively, of the 749-μg/liter, 96-h LC50 for the 8-mo-old sockeye salmon. An application factor relating 96-h LC50 and "safe" concentrations of zinc to anadromous sockeye salmon in soft water appears to be >0.15 and at least 15 times larger than the often recommended 0.01 application factor for zinc based on studies with other species.

Most toxicity tests with aquatic organisms estimate the level of effect caused by various concentrations of a pollutant. Since the effect is likely to be greater as the period of exposure is increased, the conservative approach is to conduct continuous exposure tests over a long period of time. A practical maximum period for the continuous exposure is exemplified by the full life cycle toxicity test (McKim and Benoit 1971). However, for some aquatic species, full life cycle exposures are unrealistic. The anadromous salmonids live in fresh water only a part of their lives and spend a considerable period of time in the sea. In this instance, the practical maximum period of continuous exposure to a specific pollutant is the duration of freshwater residency. However, this freshwater residency may last for several years, with an adult prespawning residency of up to 6 mo, and a juvenile residency of 1 to 2 yr which ends with the seaward migration of the smolt.

This study was conducted with Columbia River sockeye salmon (*Oncorhynchus nerka*) which usually migrate to the ocean their 2nd yr and return as adults in their 4th yr (Foerster 1968). The test was designed to determine the effect of zinc on their survival, reproduction, and growth, and on the subsequent survival of smolts transferred to seawater. The chronic zinc toxicity test lasted 21 mo, including 3-mo adult exposure, 3-mo embryo and alevin exposure, and 15-mo juvenile exposure.

METHODS

Zinc stock solutions were prepared from reagent grade zinc chloride ($ZnCl_2$) dissolved in Western Fish Toxicology Station (WFTS) well water and acidified to pH 4 with nitric acid. The toxicity test was conducted in WFTS well water which was pretreated by ultraviolet sterilization, aeration, and temperature adjustment.

Weekly chemical analyses of the well water supply were made for 21 parameters. The chemistry of the laboratory water supply is presented in greater detail elsewhere (Samuelson 1976; Chapman 1978, this issue). In addition, dissolved oxygen, total hardness, total alkalinity, pH, and zinc concentrations were routinely determined on samples from the test chambers (Table 1). Temperature was continuously recorded in each adult exposure tank and in one exposure chamber during zinc exposure of embryos and juveniles. Dissolved oxygen measurements were usually performed by the azide modification of the Winkler procedure, further modified by use of the EPA full-bottle technique and sometimes by use of phenylarsineoxide as titrant (United States Environmental Protection Agency

TABLE 1.—*Water quality characteristics*[a] *for each zinc concentration throughout the 22-mo adult-to-smolt sockeye salmon chronic toxicity test.*

Nominal zinc (μg/liter)	Measured zinc (μg/liter)	Dissolved oxygen (mg/liter)	pH[b]	Total alkalinity (mg $CaCO_3$/liter)	Total hardness (mg $CaCO_3$/liter)
0	2 (5) 183 (0–26)	9.0 (0.8) 533 (6.7–11.5)	7.2 77 (6.9–7.5)	28 (11) 77 (18–61)	35 (21) 78 (19–91)
30	30 (6) 294 (12–57)	9.2 (0.7) 511 (4.4–11.4)	7.2 77 (6.9–7.5)	28 (11) 77 (18–60)	33 (20) 77 (18–83)
60	58 (7) 297 (31–81)	9.0 (0.8) 513 (4.2–11.5)	7.2 70 (6.9–7.5)	27 (9) 70 (19–58)	32 (17) 70 (19–86)
90	87 (8) 293 (49–129)	8.9 (0.7) 511 (5.3–11.3)	7.2 74 (6.8–7.4)	30 (12) 78 (19–58)	36 (22) 78 (18–91)
120	112 (10) 296 (77–157)	9.0 (0.7) 512 (6.0–11.4)	7.2 68 (6.9–7.4)	27 (11) 69 (18–56)	33 (19) 69 (20–84)
240	242 (18) 223 (113–285)	8.9 (0.8) 539 (6.1–11.5)	7.2 57 (6.9–7.5)	30 (11) 56 (18–58)	37 (20) 56 (20–90)

[a] Above: mean (standard deviation); below: *N* (range).
[b] Above: median (all 1st and 3rd quartiles were 7.1 and 7.3); below: *N* (range).

1974). Other dissolved oxygen values were determined with a dissolved oxygen meter which was calibrated at least once daily.

The pH was determined with an Orion Model 701 pH meter.[1] Total alkalinity was determined by the potentiometric procedure for low alkalinity samples, and total hardness was determined by the EDTA titrimetric method (American Public Health Association et al. 1971).

Samples for zinc analysis were taken in acid-washed containers and were acidified by the addition of 1 ml of concentrated nitric acid per 100 ml of sample. Zinc analyses were performed by flame atomic absorption spectrophotometry on a Perkin-Elmer Model 403 with an air-acetylene flame. Absorbance was linear over the concentration range, and precision was ±3 μg/liter.

Adult Exposure

Adult sockeye salmon were exposed to zinc in a series of five identical outdoor tank systems (Fig. 1). Each system consisted of a fiberglass tank 0.9 m deep by 3.66 m in diameter for holding the adult fish; a connecting fiberglass mixing tank 0.6 m deep and 0.75 m in diameter for aeration, heating, and mixing of dilution water and metal stock solution; and a 150-liter/min water recycle system to provide additional aeration, a water current in the holding tank, and ultraviolet sterilization of the water. Standpipes maintained a water depth of 0.5 m and a volume of 5,250 liters in each exposure tank. A tarpaulin was mounted around the top edge of each tank to prevent the fish from jumping out, to provide shade, and to minimize disturbance of the fish.

Zinc stock solutions were pumped into the mixing tanks at 20 ml/min, and dilution water flow was 19 liters/min. Fifty percent replacement of tank volume took 3.3 h and 50% recycle turnover required 25 min as calculated from Sprague (1969).

On July 13, 1971, 200 adult sockeye salmon were trapped at Bonneville Dam on the Columbia River, transported to WFTS by tank truck, and individually distributed among the five test tanks by the stratified random method. Two days later all fish were anesthetized in ethyl m-aminobenzoate methanesulfonate, tagged, weighed, and measured. Thirty fish were returned to their respective tanks, while 10 randomly selected fish per tank were killed for ancillary research. The mean weight, fork length, and condition factor (weight [g] × 100/length [cm]^3) of all fish were 1.67 kg, 52.5 cm, and 1.15, respectively, and the mean loading density was 9.5 g/liter of tank volume or 1.83 g/liter/day based on the 19 liter/min flow rate.

[1] Mention of product does not constitute endorsement by the U.S. Environmental Protection Agency.

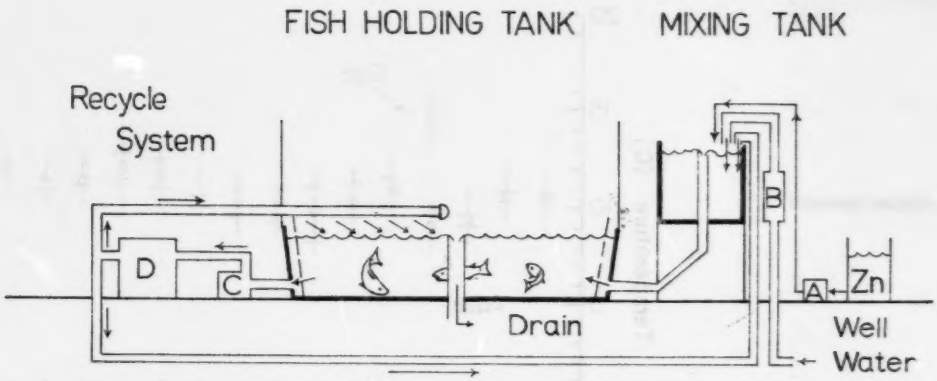

FIGURE 1.—*Schematic diagram of the adult salmon test system: (A) metering pump; (B) flow meter; (C) recycle pump; and (D) ultraviolet sterilizer.*

The fish were allowed to recover from the tagging procedure for 4 days and then exposed to nominal zinc levels of 0, 30, 60, 90, and 120 μg/liter. Four weeks later, 10 additional fish were randomly removed from each tank for ancillary research. All remaining fish were checked for maturation once in mid-September and weekly through October. Ripe adults were manually spawned from October 6 through November 2, but all eggs used for the egg-through-smolt portion of the toxicity test were from fish spawned October 13–26, and included five females from each zinc concentration and three control females. Eggs from a ripe female were collected in a graduated beaker, and 0.01 ml semen per ml of eggs was added from a ripe male from the same tank.

Embryo and Alevin Exposure

Approximately 1,300 eggs from each of three control fish and 500 eggs from each zinc-exposed fish were used in the experiment. Groups of 200 eggs from each control fish were incubated in each zinc concentration as well as under control conditions, while groups of 200 eggs from zinc-exposed fish were incubated at the parental zinc exposure level and under control conditions. Another 100 eggs per female were held in clean water for 1 wk after fertilization and were then cleared in Stockard's solution (Rugh 1952) and checked for fertility. An additional group of 25–50 eggs per female were blotted and weighed in aggregate to determine the mean weight of non-water-hardened eggs.

Embryo exposure apparatus consisted of nine trays from a Heath-Techna vertical-flow egg incubator, with each tray separately supplied with water from a proportional diluter similar to that described by Mount and Brungs (1967). Four trays received control water, and each of the five remaining trays received one of five zinc concentrations (30, 60, 90, 120, and 240 μg/liter). Each tray was divided by plexiglass partitions into 10 sections 6 × 9.5 × 5 cm deep, and mean linear water flow rate over the eggs was about 100 cm/h.

Assignment of the groups of 200 eggs was random within each tray. The lid for each tray was covered with black plastic to prevent possible damage to the developing embryos by the room lights. Once the eyes of the embryos became pigmented, unfertile eggs and dead embryos were removed, and thereafter dead embryos or alevins were removed daily.

Juvenile Exposure

The only fish held past the alevin stage were those receiving continuous exposure at control, 30-, 60-, 90-, or 120-μg/liter zinc levels through *in utero* and embryo stages, plus the fish from the 240-μg/liter embryo exposure. The surviving swim-up alevins from a common zinc treatment were pooled

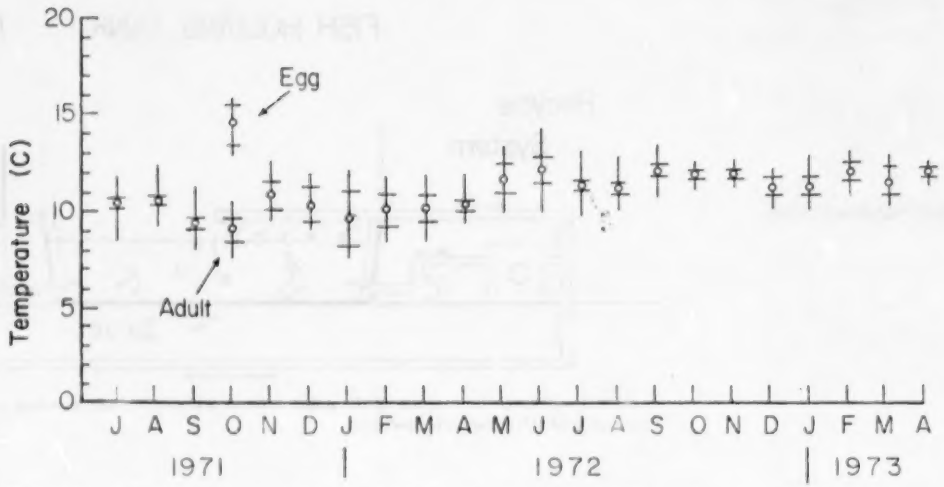

FIGURE 2.—*Mean temperature (circle); standard deviation (horizontal lines); and range (vertical line) for each month of the sockeye chronic zinc test.*

and arbitrarily divided into groups of 10 fish each. The 10-fish groups from each treatment were then randomly assigned to either of two aquaria (200 fish per aquarium) or to a surplus stock. Twenty-five fish from each surplus stock were arbitrarily selected, killed, and weighed for an initial weight sample. An additional 25 fish each from the control and 240-μg/liter surplus stocks were placed in adjacent sections of the incubator trays and exposed to measured zinc concentrations of 0, 80, 160, 240, 320, and 630 μg/liter for 115 h to determine if acclimation to zinc had occurred.

For the remainder of the experiment (January 1972 through April 1973) the fish were held in 12 aquaria (6 nominal zinc concentrations × 2 replicates/concentration) 2.5 × 40 × 30.5 cm deep with a water volume of 29 liters. Nominal zinc levels were 0, 30, 60, 90, 120, and 240 μg/liter. The aquaria were supplied with test water by a 2-liter per concentration per cycle proportional diluter similar to that described by Mount and Brungs (1967). The diluter flow for each zinc concentration was equally split between duplicate aquaria. At the diluter cycle time of 2 min, 50% turnover (Sprague 1969) occurred about every 40 min. Water in each aquarium was aerated continuously. Light intensity was approximately 500 lux at the water surface, and photoperiod, including 30 min dawn and dusk periods, was adjusted semimonthly to comply with sunrise and sunset for Corvallis, Oregon.

The fish were fed an Oregon Moist Pellet ration throughout the test, and rations were usually completely consumed. Initially, the young fish were fed 5% of their body weight in four daily feedings, but gradually the feeding rate was decreased until, at the termination of the experiment, the fish were receiving a daily ration of 1% of their body weight in a single feeding. Rations for fish in each aquarium were based on the number of fish in that aquarium and the mean blotted wet weight of sample fish from all aquaria. Starting at age 9 mo, sockeye salmon parr were fed a prophylactic diet containing oxytetracycline (terramycin, TM 50) at 0.4% of the ration for a period of 6 wk.

Ten or 20 fish were randomly selected from each aquarium for length and weight samples at 1, 2, 3, 8, and 16 mo posthatch. The fish population in each aquarium was thinned following the 8-mo weight sample because overcrowding caused ammonia levels to increase (max 0.41 mg/liter) and feeding to be lethargic. Since there were no large differences in growth among fish at

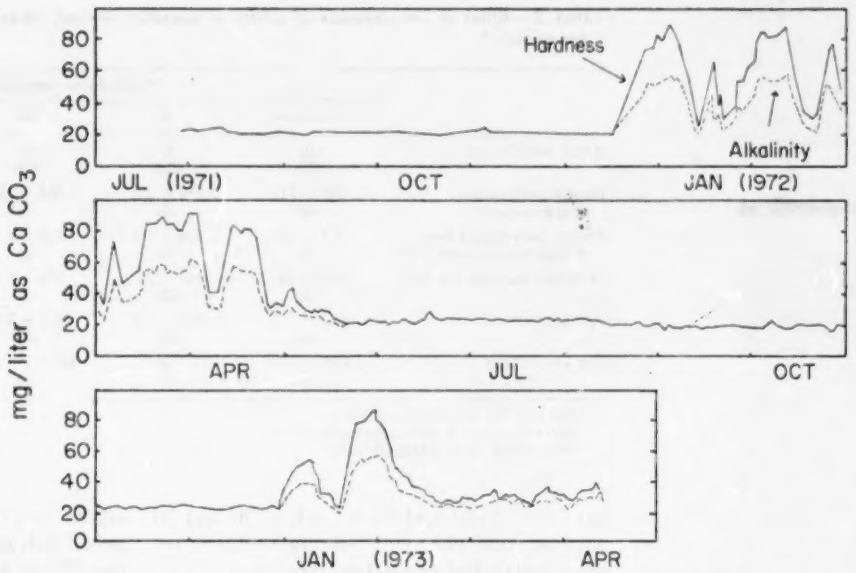

FIGURE 3.—*Alkalinity and hardness values during the sockeye chronic zinc toxicity test.*

various zinc levels, and since a considerable number of thin fish were present in each aquarium, the population thinning was done selectively rather than randomly. The 20 most robust fish were left in each aquarium while the other fish were removed and discarded or used in a 96-h acute toxicity test for zinc acclimation.

The zinc acclimation test was conducted with aquaria, diluter, and photoperiod identical to those used in the chronic zinc toxicity test. Ten fish were placed in each aquarium, with one randomly selected aquarium of each replicate pair receiving control fish and the other receiving fish from the 240-μg/liter zinc concentration. Exposure to mean measured zinc levels of 0, 245, 485, 740, 950, and 1,930 μg/liter was begun immediately and continued for 11 days. Mean water quality values during the acute toxicity test were temperature 12.4 C, dissolved oxygen 10.7 mg/liter, pH 7.3, alkalinity 21 mg/liter, and hardness 22 mg/liter. Zinc LC50 values (concentrations causing 50% mortality in a specified time) for toxicity tests with sockeye salmon alevins and parr were obtained from a computer program that linearly regressed percent mortality (probit) against metal concentration (log).

About 16 mo after hatching, the fish in the chronic exposure experiment had attained the age, size, and appearance of smolts. The fish were then transferred directly from the zinc exposure aquaria to 30‰ seawater to determine if zinc exposure had impaired the ability of the smolts to survive in seawater. The fish were starved for 48 h prior to and during seawater exposure. Five fish per aquarium were randomly selected and placed into 10 liters of aerated seawater maintained at 9 ± 1 C. Two 96-h seawater tests were conducted during consecutive weeks.

RESULTS

Water quality data for the 21-mo sockeye salmon chronic toxicity test are summarized in Table 1. Mean zinc concentrations averaged 98% of nominal, and there were no effects of zinc addition on dissolved oxygen, pH, alkalinity, or hardness. Because of aeration problems, dissolved oxygen levels fell below 6.0 mg/liter on five occasions, all during the 6th mo posthatch. This occurred three times at 60 μg/liter zinc (4.2 mg O_2/

TABLE 2.—*Effect of zinc exposure of adults on mortality, weight, fecundity, fertility, and egg weight of sockeye salmon.*[a]

	Nominal zinc concentration (μg/liter)				
	0 (control)	30	60	90	120
Adult mortality (%)	10 (20)	0 (20)	0 (20)	0 (20)	0 (20)
Female body weight at spawning (g)	1,689 ± 117 (3)	1,379 ± 207 (5)	1,462 ± 180 (5)	1,485 ± 110 (5)	1,495 ± 114 (5)
Female body weight loss during zinc exposure (%)	8.3 ± 2.4 (3)	3.4 ± 1.4 (5)	7.6 ± 1.6 (5)	7.4 ± 2.4 (5)	6.9 ± 5.7 (5)
Fecundity (ml eggs per fish)	300 ± 30 (3)	246 ± 24 (5)	276 ± 57 (5)	303 ± 33 (5)	276 ± 39 (5)
Fertility (%)[b]	99.7 ± 0.5 (3)	97.1 ± 2.4 (5)	99.6 ± 0.5 (5)	99.1 ± 1.9 (4)	99.3 ± 4.0 (5)
Egg weight (mg)[c]	99.5 ± 14.0 (3)	87.3 ± 6.7 (5)	102.7 ± 12.7 (4)	92.0 ± 4.7 (5)	93.5 ± 4.0 (5)

[a] Mean ± SD; (N) number of fish.
[b] Determined on ca. 100 eggs/female.
[c] Determined on 25–50 eggs/female.

liter minimum) and once each at 30 and 90 μg/liter zinc (4.4 and 5.3 mg O_2/liter, respectively), but caused no mortality.

Significant variation in temperature, total alkalinity, and total hardness occurred with time (Figs. 2 and 3). There was no apparent effect on the fish of the period of high water temperature (mean 14.5 C) which occurred during the first few weeks of egg incubation. Periods of elevated hardness and alkalinity occurred during both winters and may have somewhat diminished the toxic effects of the zinc.

There were no apparent adverse effects on adult sockeye salmon of any of the tested zinc concentrations. The only mortality of adults was the death of two heavily fungused fish in the control tank after 1 and 2 mo. There were no apparent differences in maturation time at any zinc concentration, and resultant fecundity, egg weight, and fertility were also unaffected by zinc (Table 2).

Differences in fecundity and egg weight among fish at various zinc levels were irregular and were apparently due to variation in female body weight and not due to zinc exposure. Regression lines for fecundity and egg weight both showed positive correlation with body weight at spawning but essentially no correlation with zinc concentration (Fig. 4). Percent body weight loss was not correlated with either body weight or zinc

TABLE 3.—*Survival rate (egg to indicated posthatch age) and mean weight of juvenile sockeye salmon reared at indicated concentrations of zinc.*

Zinc concentration (μg/liter)	Replicate	Survival (%)		Mean weight (g)	
		8 mo	16 mo	8 mo	16 mo
2	A	98.4	86.6	5.21	17.16
	B	99.0	99.0	5.31	16.54
30	A	95.6	78.7	5.38	16.55
	B	97.6	97.6	5.57	14.85
58	A	92.5	80.7	5.12	17.47
	B	91.7	91.7	5.58	18.04
87	A	96.1	95.3	5.86	17.97
	B	94.2	94.2	6.30	18.20
112	A	97.6	96.2	5.60	18.23
	B	96.5	96.5	6.38	18.39
242	A	96.1	94.5	6.20	20.06
	B	97.2	97.2	6.10	20.66

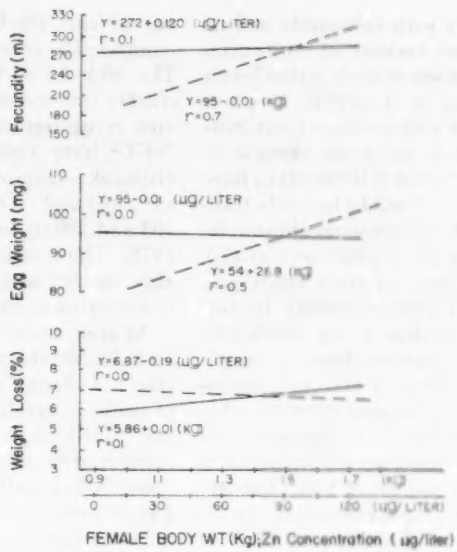

FIGURE 4.—*Regression lines relating fecundity, mean egg weight and female body weight loss to female body weight at the start of the test (dashed lines) and zinc concentration (solid lines).*

concentration (Fig. 4) so that body weight at spawning was also unrelated to zinc exposure.

Egg-to-smolt survival and growth were not adversely affected by zinc concentrations up to 240 µg/liter. Survival ranged from 88.2% at 30 µg/liter to 96.4% at 120 µg/liter, and variation in survival between replicates was often as great as that between treatments (Table 3). Growth appeared to be slightly stimulated at most zinc concentrations at both 8 and 16 mo; mean weights ranged from 5.26 g for controls to 6.15 g for 240-µg/liter fish at 8 mo, and from 15.70 g for 30-µg/liter fish to 20.36 g for 240-µg/liter fish at 16 mo. However, none of the groups was significantly heavier ($P = 0.05$) than the controls according to Dunnett's procedure (Steel and Torrie 1960).

Significant acclimation to zinc occurred and was first established in the acute toxicity test of 1-mo-old alevins in which 25 fish each from the control (nonacclimated) and 240-µg/liter exposures were exposed to zinc concentrations up to 630 µg/liter for 115 h. During the exposure, 72% of the nonacclimated alevins died at 630 µg/liter (115-h LC50, 447 µg/liter; 95% confidence limits, 385–544 µg/liter), but all of the 240-µg/liter acclimated alevins survived (LC50 > 630 µg/liter). Acclimation was demonstrated on a second occasion with flow-through acute toxicity tests of 9-mo-old sockeye salmon parr. Control fish and fish acclimated to 240 µg/liter had 96-h LC50 values (and 95% confidence limits) of 749 µg/liter (614–843) and 1,663 µg/liter (948–1,929), respectively. The degree of acclimation occurring during exposure to zinc concentrations lower than 240 µg/liter was not determined.

The final test for zinc effect was to determine if zinc exposure interfered with the survival of sockeye salmon smolts in seawater. The transfer of smolts from the various zinc concentrations to 30‰ seawater produced only one death (from 30 µg/liter) out of 120 smolts tested.

DISCUSSION

Zinc can produce deleterious chronic effects at concentrations well below acutely lethal levels. Brungs (1969) reported that fecundity of fathead minnows (*Pimephales promelas*) in hard water (200 mg/liter as $CaCO_3$) was reduced at a zinc concentration of 180 µg/liter; the 96-h LC50 was estimated at 9,200 µg/liter. Bengtsson (1974) found that growth of the minnow, *Phoxinus phoxinus*, in soft water (70 mg/liter as $CaCO_3$) was reduced at zinc levels down to 130 µg/liter; the 96-h LC50 value was estimated to be 3,200 µg/liter. The minimum chronic effect levels of zinc in these two studies were 0.02 and 0.04 of the reported 96-h LC50 values, respectively.

In the sockeye salmon zinc toxicity test no effect was seen over the entire freshwater residency period (adult to smolt) at 112 µg/liter, or over the egg-to-smolt period at 242 µg/liter. These apparently safe zinc concentrations were 0.15 and 0.32 of the 749-µg/liter, 96-h LC50 for unacclimated juvenile sockeye salmon. Thus, the 0.02 application factor (the ratio relating "safe" to lethal concentrations of zinc) for fathead minnows (Brungs 1969) appears to be considerably smaller than that (>0.15) apparently required for sockeye salmon in soft water.

There are few published data on chronic

zinc toxicity studies with salmonids and no evidence for chronic toxicity at concentrations appreciably below acutely lethal levels in soft water. Sinley et al. (1974), in a 2-yr chronic toxicity test with rainbow trout (*Salmo gairdneri*) showed significant chronic lethality (application factor 0.04–0.08) in hard water (330 mg/liter as $CaCO_3$) but only transient growth effects and minimal chronic lethality in soft water (25 mg/liter as $CaCO_3$). The remarkable aspect of their study was that acutely lethal concentrations in soft water could be lower than levels which produced no apparent chronic toxicity (application factor >1). Thus, a zinc concentration of 140 μg/liter produced no noticeable effects in a 2-yr egg-to-adult exposure, but killed over 50% of non-zinc-acclimated fish in a separate 5-day exposure test. The apparent absence of chronic effect may be attributable to the acclimation to zinc of chronically exposed fish during a relatively resistant embryonic stage.

There was no evidence in this study or in that of Sinley et al. (1974) to indicate that zinc levels below 0.32 of the 96-h LC50 would produce toxic effects on salmonids in soft water. If additional research confirms that chronic zinc exposure in soft water results in little toxicity at levels much below acutely lethal levels, then avoidance might represent a critical response of anadromous salmonids to zinc. For example, Sprague (1964) showed that Atlantic salmon (*Salmo salar*) would avoid a zinc concentration of 55 μg/liter, which was about 0.1 times the 96-h LC50.

Acute lethality of zinc is apparently reduced by increases in such water chemistry factors as hardness, alkalinity, and organic chelators. It appears that application factors for zinc might also be affected by these factors since Sinley et al. (1974) obtained a much smaller application factor in hard water than in soft water. The WFTS well water is fairly typical of many Pacific Northwest waters with respect to inorganic chemistry (Samuelson 1976), but application of metal toxicity data to water other than that actually used in the toxicity test should consider the relative metal complexing capacity of the waters in question.

The sockeye salmon was relatively tolerant of zinc (96-h LC50 = 749 μg/liter) as compared to several other salmonid species. The 96-h LC50 for cutthroat trout (*Salmo clarki*) was estimated at 90 μg/liter (Rabe and Sappington 1970) while studies at WFTS have yielded 96-h LC50 values for chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Salmo gairdneri*) of 463 and 136 μg/liter, respectively (Chapman 1978). Thus, concentrations of zinc presumably safe for sockeye salmon could be lethal to cutthroat trout and steelhead.

At zinc concentrations up to 0.32 of the 96-h LC50, there was no detectable latent effect of chronic zinc exposure on the ability of sockeye smolts to live in seawater. Lorz and McPherson (1976) reported that coho salmon (*Oncorhynchus kisutch*) that survived lethal and sublethal levels of zinc for 144 h prior to seawater exposure showed no mortality in seawater, but that similar chronic or acute freshwater exposure to copper significantly decreased subsequent survival in seawater. Thus, while some metals can produce latent mortality in seawater, such an effect may not pose a problem for anadromous salmonids exposed to zinc.

The absence of reproductive effects in the chronic zinc toxicity test with sockeye salmon may be indicative of a relative resistance of salmonids to effects such as those reported for minnows by Brungs (1969), or may be due to the 3-mo period of zinc exposure prior to spawning being too short to affect either fecundity or fertility. A final qualifying factor is that the sockeye salmon were spawned manually, precluding detection of aberrations of natural spawning behavior.

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James McKim, Gary Holcombe, and members of the WFTS staff provided technical review of the manuscript.

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